

### Remarks

Claims 13, 16, 17, 20, 21 and 23 are pending. Claims 13, 17 and 21 are currently amended. Claims 25-30 are new. Support for the amendments and new claims can be found at, for example, paragraphs [0002], [0004], [0036], [0040], [0056] and [0072] as well as the claims and sequence listing of the originally filed application. Certain of the amendments are also merely made for the sake of clarity. The Applicants respectfully request entry of the amendments and new claims which are believed to place the application in better condition for allowance or, alternatively, appeal. Claims 13, 16, 17, 20, 21 and 23 are rejected. Claims 16, 20 and 23 are cancelled and the rejections with regard to these claims are now moot.

Objections have been made to Claims 13, 17 and 21 for reciting "[a] method of decreasing intratumoral vessels" without indicating which parameter concerning the vessels is decreased. The objection indicates that amending Claims 13, 17 and 21 to recite "decreasing the number of intratumoral vessels" or "inhibiting formation of intratumoral vessels" will be sufficient to cause the withdrawal of these objections.

Amended Claims 13, 17 and 21 now contain recitations consistent with the Examiner's very helpful suggestion. Amended Claim 13 now recites that, by the steps of the method, "the number, or formation, of intratumoral vessels is decreased." Thus, the Applicants respectfully request withdrawal of the related objections to amended Claims 13, 17 and 21.

Objections have also been made to Claims 13, 17 and 21 for reciting specific desired outcomes only in the preamble without reciting that the steps of the claimed methods produce these outcomes.

Amended Claims 13, 17 and 21 now recite that the steps of the claimed methods produce the specific desired outcomes recited in the claim preambles. Amended Claim 13 now recites that, by the steps of the method, "the number, or formation, of intratumoral vessels is decreased" consistent with the preamble of this claim. Amended Claim 17 now recites that, by the steps of the method, "the number, or formation, of intratumoral vessels is decreased and melanoma in the mammal is treated" consistent with the preamble of this claim. Amended Claim 21 now recites that, by the steps of the method, "the number, or formation, of intratumoral vessels is decreased and the pulmonary metastases in the mammal are treated" consistent with the preamble of this claim. These amendments are consistent with the Examiner's helpful guidance. Thus, the

Applicants respectfully request withdrawal of the related objections to amended Claims 13, 17 and 21.

Objections have also been made to Claims 17 and 21 as being substantial duplicates of Claim 13. The Official Action cites MPEP §706.03(k) in support of the objection.

The Applicants respectfully request withdrawal of these objections to amended Claims 13, 17, and 21. First, it is well settled that applicants have a right to claim an invention in a reasonable number of ways. Second, any difference in scope between claims is enough to render claims distinct and non-duplicative. Both of these principles are articulated in the case law and MPEP §706.03(k). The Applicants respectfully note that amended Claim 13 is directed to a “method of decreasing the number, or formation of intratumoral vessels in a mammal in need thereof” while amended Claim 17 is directed to a “method of treating melanoma in a mammal in need thereof by decreasing the number, or formation, of intratumoral vessels” and amended Claim 21 is directed to a “method of treating pulmonary metastases in a mammal in need thereof by decreasing the number, or formation, of intratumoral vessels[.]” Thus, it is clear that these additional recitations render amended Claims 17 and 21 distinct from, and non-duplicative of, amended Claim 13. Third, the objection is procedurally premature as MPEP §706.03(k) teaches “it is proper after allowing one claim to object to the other [allegedly duplicative] claim under 35 CFR 1.75 as being a substantial duplicate of the allowed claim. No claims have been allowed here, so the objection is clearly premature and should be withdrawn. Altogether, the foregoing makes it clear amended Claims 13, 17 and 21 are distinct and non-duplicative and that the objection should be withdrawn as premature. Thus, the Applicants respectfully request withdrawal of the related objections to Claims 13, 17 and 21.

Claims 13, 17 and 21 have been rejected under 35 USC §112, first paragraph, as being non-enabled. The rejection states that “a method of direct administration by electrotransfer to a melanoma or a pulmonary metastasis of a nucleic acid consisting of SEQ ID NO: 1 operable linked to an expression control sequence, wherein expression of SEQ ID NO: 1 results in the decrease in the number of intratumoral vessels and in inhibition of growth of the melanoma or inhibition of the pulmonary metastases [(emphasis added)]” is enabled.

Amended Claims 13, 17 and 21 are enabled under 35 USC §112, first paragraph. Amended Claims 13, 17 and 21 now recite administering by “direct inoculation and

electrotransfer to an intramuscular site, or an intratumoral site, in the mammal a therapeutically effective amount of an expression plasmid coding for the disintegrin domain consisting of the sequence shown in SEQ ID NO: 2 where the disintegrin domain consisting of the sequence shown in SEQ ID NO: 2 is encoded by a polynucleotide sequence operably linked to a promoter or expression control sequence[(emphasis added).]” Additionally, Amended Claims 13, 17 and 21 are directed to a “method of decreasing the number, or formation, of intratumoral vessels[.]” a “method of treating melanoma in a mammal in need thereof by decreasing the number, or formation, of intratumoral vessels” and “treating pulmonary metastases in a mammal in need thereof by decreasing the number, or formation, of intratumoral vessels” respectively. The Applicants respectfully submit the amendments to Claims 13, 17 and 21 are consistent with the Examiner’s helpful guidance concerning the enablement of the claims and “direct inoculation[.]” In particular, the Applicants note the amended claims now specify the relationship between the site of administration and the treatment. Stated differently, the scope of the amended claims is consistent with the scope of enablement provided by the application, and identified by the Examiner, as well as the knowledge in the art such that undue experimentation is not required to practice the claimed methods.

However, the Applicants also wish to make a number of other comments regarding the rejection of Claims 13, 17 and 21 as being non-enabled under 35 USC §112, first paragraph.

In particular, the rejection states that “the specification teaches that the disintegrin domain of metargidin when delivered to a tumor or metastases site can cause a diminution of vessels and thus lead to a decrease in pulmonary metastases and melanoma growth” and that “for the diminution to occur, the sequence must be administered directly the the target site [(emphasis added).]” As a consequence, the rejection states it is “not clear what relationship the intratumoral site or intramuscular site have to the pulmonary metastases or melanoma targeted.”

The Applicants respectfully submit that some clarification regarding the specification and the additional data provided in the Affidavits of Mrs. Trochon-Joseph may be helpful.

First of all, the results disclosed in the Examples of the application relate to the inhibition of tumor growth by AMEP. To that end, a plasmid encoding AMEP was electrotransferred into the tibial cranial muscle of mice. See paragraphs [0087] and [0072] of the originally filed application. The tibial cranial muscle is a muscle located on the tibia bone of mice (and other

animals). Mice were then injected subcutaneously in the back with MDA-MB-231 cells, and the tumors were let to grow until reaching a volume of 18 mm<sup>3</sup>. Then doxycycline was added to the mice's drinking water to induce expression of AMEP (*i.e.*, the polypeptide of sequence SEQ ID NO: 2) in the muscles of the mice. *See* paragraph [0073] of the originally filed application. The results show that expression of AMEP led to markedly smaller tumor volumes as compared with control, and to significant inhibition of the number of vessels in the tumors. *See* paragraph [0088], Fig. 8 and Table 3 of the originally filed application.

Importantly, these results demonstrate that administration of a plasmid encoding AMEP in the tibia muscle of mice and expression of AMEP in said muscle inhibits growth and vascularisation of tumors implanted in the back of the mice. Thus, these results demonstrate the efficacy of the claimed methods even when the nucleic acid is delivered directly to a site different from the target site (*e.g.*, muscle instead of tumor site).

Second, the results disclosed in the Examples of the application also relate to the inhibition of the formation of pulmonary metastases by AMEP. To that end, C57B1/6 mice were used which had been previously injected in the tibial cranial muscle with a plasmid encoding AMEP. *See* paragraph [0072] of the originally filed application. The mice were then further injected with B16F10 mouse melanoma cells in the retro-orbital sinus, to establish pulmonary metastases. Three days after this injection of B16F10 cells, AMEP expression in the tibial cranial muscle of the mice was induced by adding doxycycline to the drinking water of the mice. *See* paragraph [0076] of the originally filed application.

Importantly, these results show that production of AMEP in the muscle of the mice inhibited the number of pulmonary metastases. *See* paragraph [0091], Fig. 9 and Fig. 10 of the originally filed application. Thus, these results again demonstrate the efficacy of the claimed methods although the nucleic acid is delivered to a site different from the target site (*e.g.*, muscle instead of site of pulmonary metastases).

This is similarly demonstrated by the data provided in the Declaration of Véronique Trochon-Joseph dated May 8, 2007, relating to the inhibition of the growth of B16F10 and C9 melanoma tumors implanted in the back of mice which were injected intratumorally (*i.e.*, directly into the melanoma) with plasmids encoding AMEP followed by electrotransfer. The results

provided showed that intratumoral electrotransfer of a plasmid encoding AMEP achieved inhibition of melanoma growth.

Furthermore, the data provided in the Declaration of Véronique Trochon-Joseph dated January 5, 2009 also illustrate that intratumoral electrotransfer of a plasmid encoding AMEP inhibited growth of B16F10 or C9 melanoma in mice. In particular, this inhibition of the growth of B16F10 and C9 melanoma was correlated with a reduction in the number of tumor blood vessels.

Altogether, the results provided in the application and in the Declarations executed by Véronique Trochon-Joseph demonstrate several important achievements. First, the Declarations demonstrate a reduction of the number, or formation, of tumor vessels can be achieved by both intramuscular, or intratumoral, electrotransfer of a plasmid encoding AMEP. Second, the Declarations demonstrate an inhibition of melanoma growth can be achieved by both intramuscular, or intratumoral electrotransfer, of a plasmid encoding AMEP. Third, the Declarations demonstrate an inhibition of pulmonary metastases can be achieved by intramuscular electrotransfer of a plasmid encoding AMEP.

Thus, the Applicants respectfully submit the application and Declarations demonstrate the claimed methods work without administering the plasmid encoding AMEP to the specific target site (*i.e.*, the melanoma or pulmonary metastases). Stated differently, the claimed methods work even if these plasmids are administered to distal sites.

In fact, expression of AMEP in the tibial cranial muscle, which is remote from the target sites, such as the back and lungs, has been shown by the Applicants to efficiently reduce the number or formation of tumor vessels, as well as inhibit the growth of melanoma tumors and development of pulmonary metastases. This is the case for the claimed methods regardless of whether others practicing different technologies may have encountered difficulties.

Therefore, contrary to the assertions in the rejection, there is no lack of clarity in the relationship between the intratumoral, or intramuscular site, of administration and the targeted pulmonary metastases or melanoma.

The rejection also alleged a high degree of unpredictability in the delivery of polynucleotides. However, the data provided in the application and the Declarations of Véronique Trochon-Joseph demonstrate that direct inoculation and electrotransfer was

successfully used to deliver several types of plasmids encoding AMEP, both intramuscularly and intratumorally.

In this regard, the Applicants respectfully direct the Examiner's attention to the data and results obtained with the pBi, pVAX1 and pORT vectors. See paragraph [0071] of the originally filed application and the Declarations dated May 8, 2007 and January 5, 2009. These data show the expression of AMEP either in muscle, or in tumors, enabled the inhibition of the number, or formation, of vessels in tumors as well as decreases in melanoma growth and the number of pulmonary metastases.

Accordingly, the Applicants respectfully submit the intramuscular, or intratumoral electrotransfer, recited in amended Claims 13, 17 and 21 does indeed achieve successful delivery of polynucleotides as evidenced by the data already present in the originally filed application and which has been entered in the application file by way of the Declarations.

The rejections also contend that animal models have not correlated well with *in vivo* clinical trial results in patients. However, the rejection also correctly notes that "human trials are not required[,] but means of enabled treatment are required." Thus, in view of the extensive data and other evidence of enablement provided by the Applicants it seems the rejections are at odds with this acknowledgement of the well settled principle that human clinical trials are not required to demonstrate enablement.

The Applicants respectfully submit the means of treatment recited in amended Claims 13, 17 and 21 (*i.e.*, intratumoral, or intramuscular, electrotransfer of a plasmid encoding AMEP) have been thoroughly demonstrated to successfully achieve the goal of the claimed methods. In particular, the Applicants have demonstrated inhibition of the number, or formation, of intratumoral vessels, the treatment of melanoma and the treatment of pulmonary metastases.

Furthermore, the Applicants also wish to note that certain of these results were obtained with melanomas established using cell lines of human origin such as MDA-MB-231 and C9 cells.

The Applicants respectfully request the withdrawal of the enablement rejections.

Claims 13, 17 and 21 have been rejected as obvious under 35 USC §103(a) over the combination of Bettan, US '408 and US '368.

Amended Claims 13, 17 and 21 are not obvious over the combination of Bettan, US '408 and US '368. Reasons are set forth below.

The rejection states that “it is obvious to combine known technologies with known products for predictable results and Bettan et al teach that it is known to administer treatment modalities on expression vectors encoding the product by electrotransfer and Merkulov [(US ‘368)] and Fanslow et al [(US ‘408)] teach that disintegrin domains provide successful therapeutic modalities.”

First of all, the alleged predictability of the result asserted in the obviousness rejections conflicts with the analysis under 35 USC §112, first paragraph, supporting the enablement rejections. The Applicants respectfully submit the Office cannot have it both ways—either neither rejection is proper due to this conflicting reasoning or at least one of the obviousness or enablement rejections should be withdrawn. Moreover, the Applicants respectfully submit that the obviousness rejection must consider all the teachings of the prior art and should be withdrawn on this basis as it ignores the teachings of unpredictability in the references relied on to support the enablement rejection.

Bettan appears to teach the intratumoral electrotransfer of plasmids encoding the luciferase or  $\beta$ -galactosidase reporter genes. In the obviousness rejection, it is asserted that “Bettan et al speak to the success of the intramuscular administration in animals.”

However, the Applicants note that each of independent Claims 13, 17 and 21 recite both “intratumoral” and “intramuscular” electrotransfer of a plasmid encoding AMEP (polypeptide of sequence SEQ ID NO: 2). This means the rejections fail to teach all the elements of the amended claims. Stated differently, the rejection fails on this basis to establish *prima facie* obviousness.

Moreover, in connection with the 35 USC §112, first paragraph, enablement rejections, the Official Action took an entirely contrary position and contended that “the method of delivery of polynucleotides is highly unpredictable to date” and cited several documents to support this analysis. Thus, the Applicants again respectfully request that all the teachings of the prior art, including those that teach away from the methods of amended Claims 13, 17 and 21, be considered.

The Applicants also had to demonstrate, based on the experimental data provided in the application and submitted in the form of Declarations executed by Ms Véronique Trochon-Joseph, that several plasmids encoding the polynucleotide having the sequence shown in SEQ ID NO: 2 were successfully delivered intramuscularly and intratumorally to achieve expression of

AMEP and produce a therapeutical benefit. Thus, the Applicants respectfully submit the incoherency between the enablement and obviousness rejections should be resolved by withdrawing both rejections.

The Applicants also respectfully submit that Bettan merely teaches that "electrotransfer not only leads to an increased number of transfected cells, but also to an increase in the number of plasmids entering each transfected cells [sic]." See Bettan at pages 87-88. The authors conclude that "electrotransfer technology could be applied with therapeutic genes to treat accessible tumors[(emphasis added).]" See Bettan at page 89. However, as acknowledged by the rejection "Bettan et al do not speak to the nature of the gene to be introduced."

The Applicants respectfully submit that two important points, contrary to the analysis in the obviousness rejections, should be made. One point is that the teachings of US '408 and US '368 would not have motivated one of ordinary skill in the art to select a gene encoding the polypeptide consisting of the sequence shown in SEQ ID NO: 2 for electrotransfer. A second point is that one of ordinary skill in the art would have had no reasonable expectation of successfully "decreasing the number, or formation, of intratumoral blood vessels" or treating melanoma or pulmonary metastases on expressing the polypeptide of sequence SEQ ID NO: 2 in muscle, or tumors, on the basis of the disclosure in US '408 and US '368.

Indeed, US '408 teaches only generally that ADAM disintegrin domains are useful for inhibiting the biological activity of integrins and for inhibiting endothelial cell migration and angiogenesis. See US '408 at column 3, lines 14-18. US '408 merely lists a group of ten ADAM disintegrin domains sequences as possible embodiments of the invention they describe. This list appears to include sequences comprising portions of the ADAM-15 disintegrin domain (*i.e.*, the disintegrin domain of metargidin) which are either larger, or smaller, than the specific, small 91 amino acid residue "disintegrin domain consisting of the sequence shown in SEQ ID NO: 2" recited in amended Claims 13, 17 and 21. This means that US '405 does not appear to disclose the specific, small 91 amino acid residue "disintegrin domain consisting of the sequence shown in SEQ ID NO: 2" let alone the uses of the composition recited in amended Claims 13, 17 and 21.

Moreover, US '408 merely appears to characterize a much larger (>240 amino acid residues) ADAM-15 disintegrin domain fused to the constant fragment (Fc) of an immunoglobulin (ADAM-15 dis-Fc) as binding to integrins  $\alpha_v\beta_3$  and  $\alpha_5\beta_1$ . See US '408 at Table

3 as well as column 17, lines 9-10 and 11-12. This means the polypeptide assayed in US '408 is not a polypeptide which is a disintegrin domain consisting of the sequence shown in SEQ ID NO: 2. US '368 is similarly deficient in this regard as it also does not teach the specific, small 91 amino acid residue "disintegrin domain consisting of the sequence shown in SEQ ID NO: 2" let alone the uses of the composition recited in amended Claims 13, 17 and 21. Bettan also fails to correct these deficiencies of US '408 and US '368. Thus, the art cited in the obviousness rejection fails to teach all the elements of amended Claims 13, 17 and 21. This also means the rejection fails to establish *prima facie* obviousness on this basis.

Moreover, prior to the Applicants' work, there was a prevailing prejudice in the art that the disintegrin domain of adamalysin alone would not be stable on expression. Importantly, the Applicants have defied the teachings in the art and unexpectedly found that the specific, small 91 amino acid residue "disintegrin domain consisting of the sequence shown in SEQ ID NO: 2" could be used successfully in the methods of amended Claims 13, 17 and 21. The Applicants submit this is compelling evidence of non-obviousness.

In support to this assertion, the Applicants point to Nath. *See* Nath *et al.*, 112 J. Cell Sci. 579 (1999) (previously submitted). Nath teaches "the disintegrin domain alone may not be stable on its own, due to the presence of an odd number of cysteine residues, which may be part of an inter-domain disulphide bond or may be involved in receptor oligomerisation (Jia *et al.*, 1997)." *See* Nath at page 581. Thus, Nath clearly teaches away from the use of the disintegrin domain alone or a disintegrin domain "consisting of the sequence shown in SEQ ID NO: 2[.]"

Additionally, in US '408, all assayed ADAM disintegrin domains were provided as fusion polypeptides with the Fc part of an immunoglobulin. *See* US '408 at column 15 and Table 2. US '408 also states that "certain polypeptides derived from antibodies are among the peptides that can promote multimerization of ADAM disintegrin domain polypeptides attached thereto[.]" *See* US '408 at column 9, lines 28-31. The Fc domain of an immunoglobulin is one such polypeptide that can promote multimerization. Thus, US '408 is actually describing a polypeptide dimer formed by dimerization of two protein subunits comprising an ADAM disintegrin domain fused to an Fc polypeptide domain capable of forming dimers or other multimers. *See* US '408 at column 9, lines 44-47.

This means US '408 did not assay the activity of an ADAM disintegrin domain alone, but instead assayed the activity of ADAM disintegrin multimers in which the disintegrin domain is fused to the Fc fragment of an immunoglobulin. Altogether, it is apparent the teachings of US '408 would not have motivated one of ordinary skill in the art to make the combination suggested by the rejection. Instead, US '408 teaches against expressing the disintegrin domain by itself, because the resulting polypeptide domain is not stable.

Furthermore, in US '408 the biological activity of the ADAM-15 Fc fusion protein molecules comprising the disintegrin domain fused to the Fc polypeptide domain (ADAM-15 dis-Fc) was evaluated *in vitro* in a PMA (phorbol-12-myristate-13-acetate) induced wound closure assay. See US '408 at column 18, lines 60-61 and Table 4. US '408 states this ADAM-15 dis-Fc fusion protein is "less effective at inhibiting endothelial cell migration" relative to ADAM-20 dis-Fc and ADAM-23 dis-Fc fusion proteins. See US '408 at column 19, lines 51-55.

As a consequence, even on hypothetically assuming one of ordinary skill in the art would have been motivated by these teachings in US '408 to select a disintegrin domain of an adamalysin, it is apparent he would have turned to an ADM-20 dis-Fc or ADAM-23 dis-Fc fusion polypeptide, rather than to an ADAM-15 dis-Fc.

US '408 also contains only prophetic examples relating to the *in vivo* activity of ADAM disintegrin domains. Indeed, Examples 5 and 6 of US '408 merely describe theoretical protocols to assay the inhibition of neovascularisation in a mouse model of cardiac transplant or to assay the treatment of tumors in an animal model. No data are provided in US '408 that could substantiate that at least one ADAM disintegrin domain within the ADAM family could provide a therapeutic benefit *in vivo* or the results of the claimed methods. Accordingly, the assertion in the rejection that the disintegrin domain of an ADAM would provide "predictable results" in terms of therapeutic benefit is not substantiated. US '408 also fails to teach all the elements of amended Claims 13, 17 and 21 because, as discussed above, it does not appear to teach the specific, small 91 amino acid residue "disintegrin domain consisting of the sequence shown in SEQ ID NO: 2" recited in the claims or that this composition can be used to achieve the results recited in the claims. In fact, US '408 is apparently silent regarding the treatment of "melanoma" and "pulmonary metastases[.]"

Additionally, US '368 only describes a protein having protease activity which comprises a large (>240 amino acid residues) amino acid sequence of the disintegrin domain of the sequence shown in SEQ ID NO: 2. US '368 does not suggest expressing a polypeptide consisting of the sequence shown in SEQ ID NO: 2 to decrease the number, or formation, of intratumoral vessels or to treat melanoma or pulmonary metastases. US '368 also fails to teach all the elements of amended Claims 13, 17 and 21 because, as discussed above, it does not appear to teach the specific, small 91 amino acid residue "disintegrin domain consisting of the sequence shown in SEQ ID NO: 2" recited in the claims or that this composition can be used to achieve the results recited in the claims. In fact, US '368 is apparently silent regarding "decreasing the number of intratumoral vessels" as well as the treatment of "melanoma" and "pulmonary metastases" as recited in the amended claims.

Accordingly, the Applicants respectfully wish to make the following important points regarding the obviousness rejections made over the combination of Bettan, US '408 and US '368. One point is that the cited combination of references fails to teach all the elements of amended Claims 13, 17 and 21. A second point is that the cited combination of references would not have motivated one of ordinary skill in the art to select a gene encoding a disintegrin domain consisting of the sequence shown in SEQ ID NO: 2 for an administration by electrotransfer. This is because the teachings of these documents would not have motivated such a person to ignore the prevailing teachings in the art against expressing the disintegrin domain alone due to the reported instability of this protein domain when expressed by itself. A third point is that, at best, the cited combination of references would have motivated one of ordinary skill in the art to express an ADAM-20 or 23 disintegrin domain fused to the Fc domain of an immunoglobulin. As discussed above, the cited combination of references would not have motivated one of ordinary skill in the art to use an ADAM-15 disintegrin domain fused to the Fc domain of an immunoglobulin. A fourth point is that the cited combination of references would have not have motivated one of ordinary skill in the art to make the combination suggested by the rejection or caused them to reasonably expect to be successful on so doing. This is because the cited combination of references provides no clue that *in vivo* expression of the disintegrin domain of an ADAM, or the disintegrin domain of metargidin (ADAM-15) consisting of the sequence shown in SEQ ID NO: 2, in particular, could be successfully used in the methods of the claims. Stated differently, the rejection fails on these bases to establish *prima facie* obviousness.

The Applicants respectfully request the withdrawal of the obviousness rejections.

In light of the foregoing, the Applicants respectfully submit that the entire application is now in condition for allowance, which is respectfully requested.

Respectfully submitted,



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